THE SIGNIFICANCE OF THE DARK PERIOD IN THE PHOTOPERIODIC RESPONSE OF MALE JUNCOS AND WHITE-THROATED SPARROWS[1]

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With a very few notable exceptions, all published studies on animal photoperiodism assign the critical role, either explicitly or implicitly, to the length of the daily period of light, acknowledging only the contributory roles of such attributes of light as wave length and intensity, and testing only such possibly modifying factors as temperature, food and activity. This conventional point of view quite disregards the existence of an accompanying, inverse variable, namely, the length of the daily period of darkness.

It is now well established that the length of the period of darkness is critically important in the photoperiodic responses of plants. So-called "long-day" plants, which flower on a relatively long daily period of light, do not *require* the long light period for flowering, since they will flower on a "short-day" if the long period of darkness defining the short day is interrupted by even a brief period of light (*e.g.*, 50 foot-candle minutes; Borthwick *et al.*, 1948).

Of the few studies on animals which incorporate data pertinent to this point, the earliest is that of Shull (1929) on aphids. He found that on a 12-hour day (12-hour night) almost all of the progeny produced by his aphids were winged, whereas on a 14-hour day (10-hour night) practically all the offspring were wingless. But he obtained the same sharply differentiated responses by exposing the aphids to 6-hour periods of light alternating either with 12-hour or 10-hour periods of darkness. Since the length of the light period was identical in the latter two cases, the difference in response must be ascribed to the difference in the length of the dark periods. In Dickson's (1949) extensive study of the photoperiodic control of diapause in the oriental fruit moth, he concluded that although the length of the light and the dark periods are both important factors, in nature diapause is caused mainly by the number of hours of darkness passing a critical minimum point. One of us (Jenner, 1951) has shown that on a short day certain snails fail to lay eggs, or lay very sparingly, but if on a shorter day the dark period is interrupted with light (total light per 24 hours equal to or less than the short-day controls), eggs are laid as abundantly as when the snails are on a long day. He has since obtained comparable results with the fresh-water shrimp, *Palaemonetes*. Hammond (1951) found that on a schedule of 7 light-hours alternating regularly with 5 dark-hours female ferrets come into oestrus ahead of ferrets exposed to normally increasing winter day-lengths; this schedule might possibly be interpreted as a combination of short days (7 hours) with short nights (5 hours), but obviously there may be other very different interpretations. Hart (1951), also using female ferrets, found that by adding

[1] An abstract of this paper appeared in the Anatomical Record, volume 113 (1952).

just one hour of light at midnight to the normally increasing day-lengths of winter the oestrus cycle was greatly accelerated. However, due to lack of an adequate control group, it cannot be determined whether his results are to be ascribed to the extra hour of light or to the interruption of the dark period.

To the best of our knowledge, there are no other published data bearing on this problem in animals, either invertebrate or vertebrate, and it is apparent that, for vertebrates at least, the evidence is not only meager but at most inconclusive.[2] The experiments to be described below were designed expressly, then, to answer the question: Is it the length of the daily period of light alone which is critical, as has been generally supposed, or does the length of the daily period of darkness play a definitive role in the photo periodic mechanism of vertebrates?

Materials and Methods

Slate-colored juncos (*Junco hyemalis*) and white-throated sparrows (*Zonotrichia albicollis*) were trapped during the winter of 1951–52 in the Arboretum on the university campus and on the grounds of the zoology building, and were distributed in six cages, six to eight birds in each cage. A 40-watt, 48-inch daylight fluorescent tube was suspended about one foot above each cage; these lights were turned on and off by automatic 24-hour clock timers. The cages were arranged in pairs, back-to-back; one cage of each pair contained juncos, the other held sparrows.

Each pair of cages was in a separate room, two of which were light-tight; in the third room the lights went on before dawn and remained on until after dark, for a total of 16 hours. The birds in this latter room are referred to as Group A (long day—short night) or long-day controls. In one of the light-tight rooms, the lights were on continuously for 10 hours in each 24; during the other 14 hours the birds were in complete and continuous darkness. These birds are referred to as Group B (short day—long night) or short-day controls. In the second light-tight room, the lights were operated by two timers in such a way that the dark period following 8¼ hours of continuous light was interrupted by a second light period of 1¾ hours. Thus, although the total hours of light per 24 were the same as in Group B, namely 10 hours, the periods of continuous darkness were only 7 hours rather than 14 hours as in B. Birds from this room are referred to as Group C (short day—interrupted night). These three light schedules are shown diagrammatically in Figure 1. The lights delivered 25–35 f.c. on the floor and perches. Group A birds were exposed occasionally to higher and more variable intensities.

The cages were constructed entirely of ¼-inch hardware cloth, and measured 2 ft. × 2 ft. × 3 ft. Each was provided with two perches made of ¼-inch dowelling, 2 feet long and placed about 5 inches above the floor of the cage, which rested on small wooden blocks.

Fine-cracked corn was present at all times in the food trays, which also contained a commercial "bird gravel." Pablum was supplied daily in a separate dish; both

[2] While this paper was in press, Kirkpatrick and Leopold (Science, volume 116, pp. 280–281) reported the induction of full sexual activity in quail held on short day—interrupted night light schedules essentially similar to those employed by us, and concluded, as we do, that "the duration of the dark period is a major controlling factor of photoperiodic responses."

species seemed to prefer this to the grain. During most of the experimental period peanut hearts were mixed with the grain, as well as small amounts of a commercial "bird seed." A continuous supply of water was assured by the use of quart jars inverted on baby-chick watering pans. The birds appeared to remain healthy under these conditions; all that were sacrificed in the course of the experiment were at least somewhat fat. Apart from a few juncos that died within 12 hours of capture, only four birds died in the cages out of a total of 38 caged birds.

Maximum and minimum temperatures were recorded daily in each room, from thermometers placed next to the floor of each pair of cages. The temperature record (° F.) is as follows:

	Average maximum	Average minimum
A	74.5 (67–81)	66.5 (60–72)
B	75.2 (72–79)	71.6 (69–76)
C	73.5 (71–77)	70.0 (67–74)

The slight differences, especially as between the control room B and experimental room C, are not regarded as significant (cf. Burger, 1948).

Groups A and B were in our personal laboratories, group C in a small, otherwise unoccupied room. To avoid disturbance to the birds the A and B cages were partly shrouded with cloth draped from the frame supporting the lights, and this effectively shielded the birds from sight of movements in the room. They did not appear to notice the sound of conversation or other noises; the birds of group A, especially, frequently sang throughout the day. Since the results place group B in sharp contrast with both A and C, the isolated and relatively undisturbed position of the C cages is not regarded as relevant.

Juncos were trapped on January 4, 5 and 6, 1952, banded and placed under treatment on the dates of capture. All the juncos were killed on March 1, after 55–57 days of treatment. The initial group of white-throated sparrows was trapped during the period December 17–20, 1951, and the experiment begun on December 19. Additional sparrows were trapped and added to the cages between December 30 and January 3. These latter birds were banded to distinguish them from the original, unbanded lot, but the loss of some bands in the C cage made uncertain the length of treatment for the first C birds killed, on January 17. The sparrow experiment was terminated on February 21. Birds were classed as adults or immatures on the basis of the frontal bones (Miller, 1946) at the time of sacrifice. It is possible that some which were "immatures," *i.e.*, birds of the year, at the start of the experiment had become "adults" on this criterion by the end of the experimental period.

The birds were killed with chloroform, in groups of three to six. Both testes of each male were removed with fine forceps, placed together in a tared weighing bottle, weighed on a balance to the nearest 0.1 mg. and then immediately flooded with Bouin's fluid. Long and short diameters of each testis were subsequently measured for determination of volumes, and one (usually the larger) of each pair was sectioned at 10 μ; the sections were stained with Ehrlich's hematoxylin, some counterstained with eosin. The volumetric data in Tables I and III refer to the sectioned testis, weights to the pairs of fresh testes. Maximum tubule diameters were measured from the sections with an ocular micrometer, and the progress of spermatogenesis recorded according to the stages of Blanchard (Blanchard, 1941;

TABLE I

Some data on results of artificially imposed lengths of day and night on male slate-colored juncos (Junco hyemalis)

Date collected (1952)	Duration of treatment (days)	Weight both testes (mg.)	Volume sectioned testis ($mm.^3$)	Max. tubule diam. (μ)	Stage of spermatogenesis
Field Controls					
Jan. 6	—	1.6	0.64	55	I
Jan. 8 (immature)	—	0.6	0.31	52	I
Jan. 11 (immature)	—	0.4	0.19	42	I
Feb. 28	—	4.7	2.05	100	IV
Feb. 28	—	1.4	0.47	75	II
Mar. 5	—	1.2	0.68	65	II
Mar. 6	—	1.9	1.06	82	III
Mar. 6	—	2.3	0.82	78	III
Mar. 6	—	1.8	0.74	75	III
Mar. 6	—	1.4	0.74	88	III
Mar. 6	—	0.6	0.58	82	III
Mar. 6	—	0.8	0.43	75	III
Group A. "Long day—short night"					
Jan. 4	57	348.8	205.0	575	VII
Jan. 4	57	188.5	93.0	525	VII
Jan. 5	56	245.2	139.0	600	VII
Jan. 5	56	160.1	94.0	500	VII
Jan. 6	55	184.8	107.0	450	VII
Group B. "Short day—long night"					
Jan. 4	57	0.9	0.52	78	III
Jan. 4	57	0.3	0.23	58	II
Jan. 5	56	1.6	0.76	91	III
Jan. 5	56	0.7	0.38	72	II
Jan. 6	55	0.8	0.42	65	II
Group C. "Short day—interrupted night"					
Jan. 4	57	128.5	70.5	400	VII
Jan. 4	57	76.2	37.5	350	VI
Jan. 4	57	50.8	25.5	300	VI
Jan. 5	56	77.7	42.5	375	VI
Jan. 5	56	4.2	1.84	125	IV
Jan. 6	55	18.2	8.10	200	V

Blanchard and Erickson, 1949). These stages may be characterized, briefly, as follows:

I. Inactive; interstitial lipoid Leydig cells absent, or, if present, small, not recognizable in ordinary preparations.
II. First appearance of recognizable Leydig cells.

III. Marked increase in number of spermatogonia; Leydig cells numerous.
IV. A few primary spermatocytes in synapsis in each tubule cross-section.
V. Predominance of primary spermatocytes in synapsis.
VI. (early). Predominance of spermatids.
VI. (late). Spermatids in transformation, much cellular debris in lumen of tubule, many sperm bundles but fewer sperm per bundle than in fully mature condition.
VII. Breeding condition; tubules packed with sperm bundles; mature sperm free in lumen.

Results

Slate-colored juncos (Junco hyemalis). (Fig. 2; Tables I and II). The field controls demonstrate that at the beginning of the experiment the testes were minute and spermatogenically inactive.

Group A. After 8 weeks on a "long day—short night" schedule, spermatogenesis was complete in each of the five males in this group. The tubules were swollen, each with a large lumen, and packed with bundles of mature sperm.

Group B. Recrudescence of the testis was initiated in each of the five males on a "short day—long night" schedule after 8 weeks. However, none had developed beyond the stage of marked increase of spermatogonia (stage III) and only two had even reached that stage. The testes were still minute, but the tubules had enlarged slightly.

Group C. After 8 weeks on the experimental "short day—interrupted night" schedule, testes of four of the six males contained fully developed sperm in numerous bundles. One of these four corresponded to the long-day group A testes, with spermatogenesis complete; in the other three many spermatids were still in the process

Table II

Stages of spermatogenesis attained by slate-colored juncos, artificially lighted from Jan. 4–6 to Mar. 1. Each "x" indicates one bird

Stages	Field Jan. 6–11	A Long day	B Short day	C Interrupted night	Field Feb. 28–Mar. 6
VII		xxxxx		x	
VI late				xxx	
VI early					
V				x	
IV				x	x
III			xx		xxxxxx
II			xxx		xx
I	xxx				

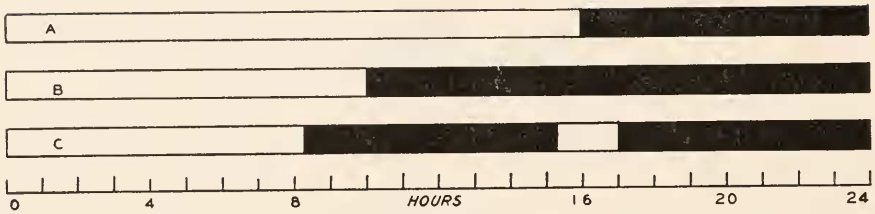

FIGURE 1. Schematic diagram of light schedules per 24 hours. A = long-day control; B = short-day control; C = "interrupted night." Total number of light hours in C the same as in B, but through interruption of darkness in C, periods of continuous darkness are one hour less than in A.

of metamorphosis, although there were many small bundles of metamorphosed sperm in each tubule section. In the testes of the remaining two experimental birds the first maturation division was under way.

Most of the juncos field-trapped and sacrificed at about the termination of the experiment were in spermatogenic states comparable to those attained by our short-day controls, group B. Only one of nine had reached the stage of initiation of spermatocyte division. That one is thus similar to the least developed of our experimental birds of group C. At this time, day lengths were approaching 12 hours (sunrise-sunset), and these outside birds had been exposed, for almost four weeks, to day lengths exceeding the 10 hours allowed our experimental and short-day control birds.

White-throated sparrows (*Zonotrichia albicollis*). (Tables III and IV.) The results, at first glance, may not appear to be as convincing as in the case of the juncos.

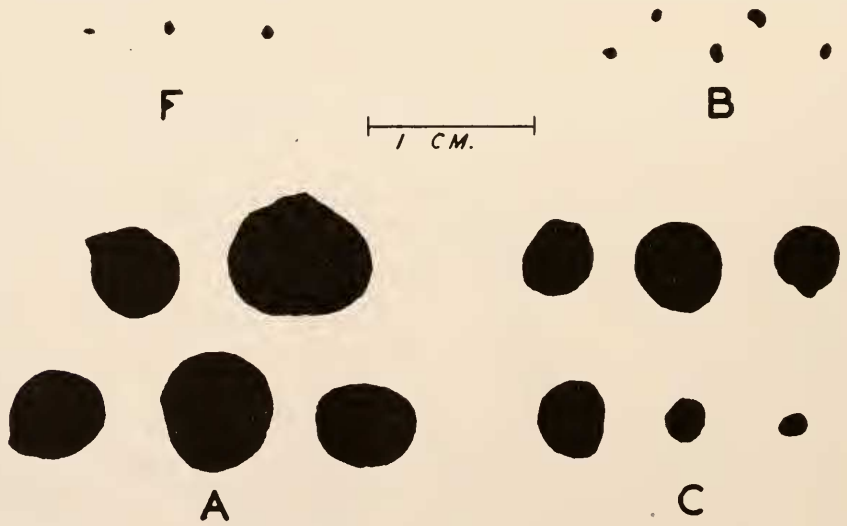

FIGURE 2. Relative sizes of junco testes in early January (F = field controls) at start of experiment, and after 55–57 days on the experimental light schedules of Figure 1; each testis is from a different bird. From a negative photographic print. A = long-day control; B = short-day control; C = "interrupted night."

Most of the birds were sacrificed at the end of four weeks, a date which proved to be perhaps too early, and left very few birds to be treated for seven weeks. Then, due to the loss of some bands, the group C birds sacrificed at the end of four weeks included a few undetermined individuals that had been in the cages only 15–18 days. Nevertheless, we believe, especially in the light of the results with juncos, that these

TABLE III

Some data on results of artificially imposed lengths of day and night on male white-throated sparrows (Zonotrichia albicollis)

Date collected (1951–52)	Duration of treatment (days)	Weight both testes (mg.)	Volume sectioned testis ($mm.^3$)	Max. tubule diam. (μ)	Stage of spermatogenesis
		Field Controls			
Dec. 29	—	1.9	1.01	58	I
Dec. 29	—	2.1	0.98	55	I
Dec. 29	—	2.2	0.89	55	I
Dec. 29 (immature)	—	0.5	0.42	46	I
Feb. 28	—	4.0	3.04	68	II
Feb. 29	—	5.5	2.35	65	II
Feb. 29	—	0.6	0.55	49	I
		Group A. "Long day—short night"			
Dec. 17–20	29–30	161.0	87.0	420	VII
Dec. 17–20	29–30	100.9	47.9	330	VI
Dec. 17–20	29–30	81.0	31.4	390	VI
Dec. 30	52	274.1	200.0	650	VII
Jan. 1	51	151.5	67.5	520	VII
Jan. 3	49	258.7	143.5	600	VII
		Group B. "Short day—long night"			
Dec. 17–20	29–30	2.7	1.20	68	II
Dec. 17–20	29–30	2.7	1.06	65	I
Dec. 17–20	29–30	1.9	1.06	71	II
Dec. 17–20	29–30	1.9	0.95	62	I
Dec. 17–20 (immature)	29–30	—	1.08	65	I
Jan. 1	51	0.5	0.34	58	II
Jan. 3	11*	1.9	0.95	62	II
		Group C. "Short day—interrupted night"			
Dec. 17–20	29–30	5.6	3.32	81	III
Dec. 17–20	29–30	3.8	1.20	62	II
Dec. 17–20 (immature)	29–30?	2.3	0.95	81	III
Dec. 17–20 (immature)	29–30	1.9	0.78	71	II
Dec. 17–20 (immature)	29–30	1.2	0.84	68	II
Jan. 3	49	52.1	31.2	290	VI
Jan. 3	49	9.4	10.3	160	IV

* = died in cage; ? = some of these collected Dec. 20–Jan. 3, treated only 15–18 days (bands lost).

data for white-throated sparrows do show that the interruption of the long dark period resulted in an acceleration of the spermatogenic process. At the beginning of the experiment (field controls), the testes of all birds were minute and inactive.

Group A. On a "long day—short night" schedule, the testes of three birds had greatly increased in size after four weeks. In one of these the tubules were packed with sperm, as in the breeding condition; in the other two the tubules were greatly enlarged, with many spermatids and a few sperm. After 7 weeks, the testes of three additional males had reached full breeding condition.

Group B. On the "short day—long night" regime, after four weeks, the testes of the three most advanced males showed just the beginning of recrudescence; in three others, the testes still were inactive. Even after 7 weeks, the single male left in this group had not advanced beyond the earliest stage of recrudescence (first appearance of interstitial Leydig cells).

Group C. Of the experimental males on a "short day—interrupted night," after four weeks the testes of one were definitely enlarged, and spermatogenesis had reached the stage of marked increase of spermatogonia. In another male the testes were still small, but the tubules showed a definite although slight increase in diameter and spermatogenesis had likewise reached the multiplication stage (III). The other three males had begun recrudescence. No testis was still in the inactive state, despite the fact that some of these birds (individuals unknown) had been treated for only 15–18 days. Two males were treated for 7 weeks; in both of these the testes had increased very considerably in size. One had reached the penultimate stage of spermatogenesis, with swollen tubules packed with spermatids; in the other some spermatocytes were beginning to divide. This latter condition is the one

TABLE IV

Stages of spermatogenesis attained by white-throated sparrows under artificial lighting

Stages	Field Dec. 29	A Long day	B Short day	C Interrupted night	Field Feb. 28–29
VII		a bbb			
VI late					
VI early		aa		b	
V					
IV				b	
III				aa	
II			aaa b	aaa	xx
I	xxxx		aaa		x

"a", "b" and "x" each denote one individual; "a" = experimental period of 29–30 days (Dec. 19–20 to Jan. 17), and "b" = experimental period of 49–52 days (Dec. 30–Jan. 3 to Feb. 21).

which, in a related species, either immediately precedes the beginning of spring migration, or, in non-migratory populations, accompanies pairing and onset of territorial behavior (*Z. leucophrys*, Blanchard, 1941; Blanchard and Erickson, 1949).

It is unfortunate that only one male was left in the control group B after seven weeks; but it is noteworthy that several days after the termination of the experiment three males collected in the field were far behind the less mature of the last two experimental birds. As explained above under juncos, the outside birds had been exposed to considerably more light per 24 hours than our groups B and C.

Discussion

Among our birds on the short day—interrupted night schedule, full testicular development was obtained not by exposing the birds to extra light but by breaking the dark period into two "short nights." It is therefore concluded that the length of the uninterrupted period of darkness is a controlling factor. Darkness evidently is not neutral or merely indifferent in the activation or inactivation of the photoperiodic response. Some sort of reaction must be going on in the dark which, probably, bears an inverse relationship to the reaction going on in light. If the dark period is long enough gonadotropic activity does not occur. If the dark period is short (or absent) gonadotropic activity does occur, but its occurrence is independent of the *length* of the light period, providing only that the duration of the light period is greater than some relatively low minimum. The significance of the length of the photoperiod is related primarily to the way in which it defines the length of the dark period. The species used in our experiments, and undoubtedly some other passerine birds as well, certainly do not require at least 11 to 12 hours daily light to complete spermatogenesis, as the conventional view would have it. What they do require, apparently, is that the daily dark period be less than 12 or 13 hours.

In our experiments the short day—interrupted night schedule obviously was not as effective in stimulating spermatogenic advance as was the long day—short night schedule, even though the dark periods (7 hrs.) were shorter than the dark period (8 hrs.) of the long day. Further investigation will be necessary to explain this difference. In our opinion the probable explanation is that the reaction which occurs in the light reaches a maximum at a quantity of light (intensity × time) greater than that employed for the dark-period interruption in our experiments. The fact that the testes of four of the six juncos on the interrupted-night schedule developed mature sperm (late stage VI or VII) in 8 weeks would seem to indicate that the interruption given (1¾ hours at about 30 f.c.) approached the light energy required for complete effectiveness. Therefore, if we consider that a light quantity slightly greater than 1¾ hours at 30 f.c. represents the order of magnitude of the light energy required to complete the light phase of the photoperiodic response, then it is obvious that in our experiments the light reaction reached the same maximum on the short day of 10 hours (group B) as on the long day of 16 hours (group A). The difference in response between these two day lengths must then be due to the difference in the length of the dark periods. We interpret this to indicate that whereas the light-phase of the reaction reaches a maximum rapidly, the dark-phase reaction takes place very slowly, and is therefore the time-measuring phase of photoperiodism.

There is an extensive literature on photoperiodism in birds and mammals. Conventionally it is thought that in these responses ". . . the light is received by the eye which sets off an unknown sequence of events terminating in stimulation of the pituitary" (Burger, 1949; p. 218). The pituitary in turn produces the gonadotropins which stimulate the gonads. The experiments reported here direct attention to what appears to be a characteristic feature of all photoperiodic responses, namely, that the duration of the dark period is critically important. The conventional view of animal photoperiodism must therefore be amended to include this concept.

The results of the present experiments, like those of Jenner (1951), demonstrate a similarity between plants and animals in their photoperiodic responses. The recognition of such a pattern of similarity emphasizes the desirability of understanding its basis.

Summary

1. Experiments were conducted with slate-colored juncos and white-throated sparrows to test the thesis that the dark period plays a definitive role in the photoperiodic response of these birds.

2. The results of the experiment using male juncos were as follows:

(a) Testes of birds on a short day (10 hours of light, 14 hours of darkness) underwent little or no development during the 8 weeks of the experiment.

(b) Exposure of other juncos to a long day (16 hours of light, 8 hours of darkness) during this same period resulted in their testes attaining full breeding condition.

(c) Experimental birds were placed on a light schedule which consisted of 8¼ hours of light followed by a dark period, the middle of which was interrupted by a second light period of 1¾ hours; thus they received a total (as in (a)) of 10 hours of light per 24-hour period, but dark periods were only 7 hours long. By the end of 8 weeks considerable testicular development had occurred among all males placed on this "interrupted-night" schedule. The testes of four birds had developed mature sperm (late stage VI or VII); the other two were somewhat less developed.

3. The response of white-throated sparrows placed under these same conditions was similar to that of the juncos.

4. The results show clearly that in the photoperiodic response of these birds there is a critically important dark-dependent phase. This dark-period dependence appears to be characteristic of all photoperiodic responses, in both plants and animals.

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